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APOGAMY IN NEPHRODIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 109

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(WITH PLATES IX AND X, AND THREE TEXT FIGURES)

Introduction

The term apogamy was proposed by DE BARY in 1878, following
FARLOW's discovery (21, 22) that in *Pteris cretica*, under artificial
culture, the sporophyte is developed from the gametophyte with the
suppression of the sexual act. Since this discovery, the apogamous
development of a sporophyte as a vegetative outgrowth from the
gametophyte in pteridophytes, together with the phenomena of par-
thenogenesis, where the sporophyte is developed from an unfertilized
egg, has been described in many forms.

FARLOW (22), in contrasting the apogamous embryo with the
normal one, notes the following four points: (1) the apogamous
embryo is intimately connected with the prothallium in such a way
that one cannot decide where the one begins and the other ends;
(2) there is formed no foot or equivalent organ; (3) the vascular
bundle of the sporophyte is in direct connection with vessels which lie
wholly in the prothallium; (4) the order of evolution is different, a
leaf arising first and becoming tolerably well developed before the
root and afterward the stem make their appearance.

FARLOW's investigation was followed by an extensive study of
DE BARY (1) on a number of forms in Polypodiaceae, in which he
described a similar sporophytic growth in *Aspidium Filix-mas
cristatum* and *A. falcatum*. He records various conditions of the
development of sexual organs in apogamous prothallia: in *Aspidium*

Filix-mas cristatum archegonia are apparently absent, in *Pteris cretica* they never fully developed; although all prothallia bear more or less numerous antheridia.

SADEBECK in the following year reported apogamy in *Todea africana* (in SCHENK's *Handbuch der Botanik* **1**:233. 1879). And later apogamy was found in *Osmunda regalis* and *Ceratopteris* (LEITGEB **38**); *Todea rivularis, T. pellucida* (STANGE **63**); *Doodya caudata* (STANGE **63,** HEIM **29**); *Trichomanes alatum* (BOWER **6**); *Selaginella rupestris* (LYON **43**); *Trichomanes Krausii, Pellaea flavens, P. nivea, P. tenera, Notochlaena Eckloniana, N. sinuata, N. Marantae, Gymnogramme farinifera* (WORONIN **78, 79**); and in some others.

LANG's study (**36**) of the apogamous development of the sporophytes on prothallia of several forms of Polypodiaceae is the most detailed contribution on apogamy in pteridophytes. The paper presents a discussion of the phenomenon in relation to alternation of generations, and adds detailed descriptions of the results of cultivating prothallia grown from spores, for a period of two years and a half in the following fourteen forms: *Aspidium aculeatum* Sw. var. *multifidum* Woll, *A. angulare* Willd. var. *foliosum multifidum*, var. *acutifolium multifidum* (no apogamy seen), *A. frondosum* Lowe; *Athyrium nipponicum* Mett., var. *cristatum*, *A. Filix-foemina* Bernh. · var. *percristatum* Cousens, var. *cruciato-cristatum*, var. *coronatum* Lowe; *Nephrodium dilatatum* Desv. var. *cristatum gracile*, *N. Oreopteris* var. *coronans* Barnes; *Polypodium vulgare* L. var. *grandiceps* Fox; *Scolopendrium vulgare* Sm. var. *ramulosissimum* Woll, var. *marginale*.

According to his account, the apogamous growth resulted from artificial cultures, watered entirely from below and exposed to direct sunlight, important departures from the normal conditions surrounding fern prothallia. The asexual sporophytic outgrowth from the prothallia present some minor differences in different individuals, but, taken as a whole, they arose in the following ways: as leaves, roots, and ramenta directly on the prothallia or on a cylindrical process from the prothallium; as a continuation of the process as a leaf; as sporangia on the process from the prothallium; as tracheids in the prothallia or in the middle lobe and cylindrical process.

It seems hard to draw from these experiments any more precise

conclusion than that the normal life-history is checked at a critical period (fertilization) and that the plant is forced into an expression of vegetative activity. Apogamy brings forward also the theory of the homologous origin of alternation as contrasted with the antithetic. The theory of the homologous origin of the alternation of generations, as held by PRINGSHEIM (**55, 56**) and SCOTT (**61**), is discussed by LANG in his studies on apogamy. He is inclined to the opinion that apogamy and apospory in ferns support the homologous view, since the prothallium can so readily take on sporophytic characters and the sporophyte can develop the gametophyte aposporously. He recognized, however, that all speculations on these points must be tentative until the actual nuclear conditions in apogamy and apospory have been ascertained.

The investigations mentioned above have greatly extended our knowledge concerning the phenomena of apogamy and apospory, and some of them have contributed much to elucidate the structural features involved, but cytological details of apogamy in pteridophytes remained unknown until last year, when there appeared two papers, one by FARMER and DIGBY (**24**) and the other by STRASBURGER (**68**).

FARMER and DIGBY'S paper is one of the most important of recent contributions to apogamy in ferns. Their preliminary note (**23**), published four years ago, announced the discovery of nuclear fusions in the vegetative cells previous to the apogamous sporophytic outgrowths from prothallia. The final paper deals with the results of their studies on apogamy and apospory in the following seven forms: *Lastrea pseudo-mas* vars. *polydactyla* Wills., *polydactyla* Dadds, *cristata apospora* Druery; *Athyrium Filix-foemina* vars. *clarissima* Jones, *clarissima* Bolton, and *unco-glomeratum* Stansfield; and *Scolopendrium vulgare* var. *crispum Drummondae*.

All the prothallia of the five forms used for their investigations, excepting the two *polydactyla* varieties, were aposporus outgrowths, either on peripheral cells of unmatured sporangia, from sori of sterile sporangia, or on apices, surface, or margins of pinnae. In two forms —var. *unco-glomeratum* and var. *cristata apospora*—the aposporous prothallia were induced artificially by pegging detached pinnae down on damp soil. The two varieties *polydactyla* of Lastrea, producing ordinary spores and prothallia, were obtained by sowing the spores.

In these apogamous prothallia antheridia are always produced in profusion and sperms are matured in every case, but the development of archegonia is different in different forms: sometimes no archegonia are produced (var. *polydactyla* Wills. and *cristata apospora*), or some are formed but do not attain maturity (var. *clarissima* Jones), and in these two cases sporophytes appeared apogamously as vegetative outgrowths; in other cases an egg is produced in the normal way, but there is no fertilization, the embryo being developed either from the unfertilized egg (var. *clarissima* Bolton and Scolopendrium) or endogenously in connection with an archegonium (*unco-glomeratum* Stansfield).

So far as the number of chromosomes is concerned, these seven cases of apospory and apogamy may be placed in three categories: (1) in the two vars. *polydactyla*, in which sporogenesis shows the ordinary reduction, a doubling of chromosomes is attained by the fusion of vegetative nuclei instead of by fertilization; (2) in four forms—three varieties of *Athyrium Filix-foemina* and a Scolopendrium—sporogenesis is omitted from the life-cycle, prothallia arise directly from abortive sporangia or pinnae, and sporophytes develop apogamously from the prothallia, the approximate sporophytic number of chromosomes being retained throughout the cycle; (3) in var. *cristata apospora*, in which apospory and apogamy regularly follow each other, the approximate number of chromosomes through the life-cycle is 60, and the authors believe that in this case the sporophyte may retain the original gametophytic number of chromosomes, the suggestion being based upon the fact that the number 60 approximates 72, the gametophyte number of the type species *Lastrea pseudo-mas*.

Throughout the investigation the authors met a variable number of chromosomes in different parts of an individual, and they express the belief that such variations are not due entirely to errors of counting, but that they correspond to fluctuations in the number actually present in the different regions. Their general conclusions are as follows: there is no necessary correlation between the periodic reduction in the number of chromosomes and the alternation of generations. Fertilization and reduction, however, are recognized as holding a very definite relation to one another, but without any *a priori* grounds for assuming any necessary connection between either of them and any other

feature in the life-history; and therefore the problem of alternation must be settled by an appeal to evidence other than that derived from the facts of mitosis.

STRASBURGER (**68**) studied apogamy in Marsilia from the cytological standpoint. Previous to his work, parthenogenesis in Marsilia had been described by two observers. Almost ten years ago SHAW (**62**) found normal parthenogenesis, as it was called by the author, to be of frequent occurrence. He also isolated megaspores of *Marsilia Drummondii* from microspores before the sperms matured, and over 50 per cent. of the isolated female prothallia produced embryos, while not more than 69 per cent. of those which were mixed with male prothallia produced embryos. Four years later NATHANSOHN (**48**) induced parthenogenesis in *Marsilia vestita* and *M. macra*. He found that it was possible to stimulate the egg cell to a parthenogenetic development by exposing the germinating megaspores to a temperature of 35° C. for 24 hours, and allowing them to continue their development at a temperature of 27° C. As a result, about 7–12 per cent. of the spores gave rise to parthenogenetic embryos. Under lower temperatures the egg developed an embryo only after fertilization. No nuclear conditions were noted by SHAW or NATHANSOHN.

In parthenogenesis in seed plants, a reduction of chromosomes does not occur in the formation of the embryo sac, so that the egg nucleus contains the diploid number, which naturally obviates any necessity for the act of fertilization. Considering parthenogenesis in seed plants, there arises naturally a question as to how the egg nucleus in Marsilia, which usually establishes a new sporophyte after a normal act of fertilization, could have developed parthenogenetically into the sporophyte. A clear explanation of this question from the cytological standpoint was made by STRASBURGER in his recent paper (**68**).

STRASBURGER states that the classification into species in Marsilia cannot always be relied upon. He finds that megaspores of BRAUN's *Marsilia Drummondii* develop embryos habitually by parthenogenesis, and that in three species—*M. vestita, M. aegyptica,* and *M. quadrifolia*—embryos are formed only after fertilization.

In *Marsilia Drummondii* he finds that the nuclei of the prothallia in the megaspores contain 32 chromosomes, the diploid number, as found in the root tips and other vegetative structures of the sporophyte.

One or two ventral canal cells always persist without disorganizing, which may also prevent the entrance of sperms and check the act of fertilization.

In *Marsilia vestita*, the species worked by NATHANSOHN, STRAS-BURGER tried NATHANSOHN's method of inducing parthenogenetic growth, but did not obtain a single parthenogenetic embryo. In this species the sporophytic number 32 is reduced to 16 in sporogenesis, and hence under natural conditions an embryo should develop only after fertilization. He found similar normal conditions in *M. quad-rifolia*, *M. elata*, and *M. hirsuta*, whose chromosome number is the same as that of *M. vestita*.

According to his detailed account of sporogenesis in *Marsilia Drummondii*, the number of megaspore mother cells is less than 16, the usual number in normal forms, and sometimes only four. In diakinesis 32 chromosomes appear, and in the metaphase there were observed two kinds of mitotic figures, one of heterotypic type with 16 bivalent chromosomes, and the other of vegetative type with 32 univalent chromosomes. In both cases a second division follows, so that two kinds of megaspore tetrads are formed, one with the $2x$ or diploid number of chromosomes, and the other with the x or haploid number. The proportion of these two kinds of spores differs in each individual form; for instance, in GOEBEL's material he found the megaspores with the diploid number only. In micro-sporogenesis there was observed a tendency toward forming a hetero-typic figure, but no mature sperms developed; two species, *M. macra* and *M. Nardu*, behaved similarly.

Such a megaspore forms a prothallium whose nuclei have the diploid number of chromosomes, which pass to the egg nuclei, so that the sporophytic number of chromosomes is maintained through-out the life-history, as in cases of parthenogenesis known among seed plants; however, the case found in *Marsilia Drummondii* by STRAS-BURGER, where a tetrad division occurs not accompanied by chromo-some reduction, seems to be a condition never described before, because most of the cases of parthenogenesis known in seed plants are characterized by the omission of the tetrad division as well as the accompanying reduction.

STRASBURGER calls the phenomenon in Marsilia apogamy, main-

taining the principle stated in his paper on parthenogenesis in EUAL-
CHEMILLA (66), that the asexual development of an embryo from the
gametophyte with the diploid number of chromosomes, whether the
embryo comes from an egg or a vegetative cell, should be regarded
as apogamy; while the term parthenogenesis should be reserved for
the asexual development of an egg with the haploid number of chromo-
somes and consequently capable of being fertilized.   WINKLER (76)
opposed STRASBURGER'S view concerning the application of the terms
apogamy and parthenogenesis.   The difference of opinion concerns
not only the question of terminology, but also involves theoretical
views regarding the significance of the number of chromosomes,
which will be considered later.

The present investigation on apogamy in *Nephrodium molle*
Desv. was undertaken in the hope of adding something to our knowl-
edge concerning the cytological interpretation of the phenomenon of
apogamy.   As was stated in the preliminary note, the writer was
convinced of the necessity of understanding beforehand the nuclear
conditions throughout the whole normal life-history of this species.
Consequently, first sporogenesis, and second spermatogenesis, oogene-
sis, and fertilization were studied.   These results were published in
two preceding papers (81, 82).

Material and methods

All of the apogamous prothallia used in this investigation were
raised from ordinary spores, secured from the same material used for
the study of sporogenesis, which was collected from the greenhouses of
the Hull Botanical Laboratory, of Lincoln Park, and of Washington
Park, Chicago.

Spores were sown upon sterilized soil consisting of a mixture of
vegetable mold and sand, placed in the greenhouse, and kept grow-
ing with special care since October 1906.   Some of the prothallia
presented certain peculiarities, one being different from the rest in
the same pots, but in general the differences held no relation to
apogamous development.   Antheridia and archegonia were pro-
duced in profusion.   Nuclear conditions in the vegetative cells, as
well as in spermatogenesis, oogenesis, and fertilization, were studied

in this material and the results were published in the preceding papers (**81, 82**).

In a number of pots placed in saucers filled with water like the rest, watering from above was avoided and the cultures were exposed to direct sunlight after the prothallia had developed two or three cells. The excessive evaporation from the soil was regulated carefully, so as not to permit condensation on the prothallia, and allow fertilization. Thus the prothallia were kept growing for a long period in dryness and in exposure to direct sunlight, the temperature of the room being kept at 28°–32° C. No fungi or lower algae developed in the pots.

The rate of growth of these prothallia when compared with that of those placed in normal conditions was quite slow. Antheridia appeared earlier than under normal conditions and were very numerous. About five or six weeks after the prothallia of two or three cells were examined, there was observed a peculiar thickening in the cushion region of some of the prothallia which reached the cushion condition earlier than the rest. This thickening was determined afterward to be the initiation of an apogamous sporophytic outgrowth. During the next three or four weeks the growth of the sporophyte was rather rapid, and at the end of that time it had become leafy. Fixation of the prothallia was made during all stages of development.

The killing and fixing of the material, with washing, imbedding, cutting, and staining, were done by the method used in the study of spermatogenesis, oogenesis, and fertilization.

This investigation was begun in October 1906, at the suggestion of Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN, and I wish to express my sincere gratitude to these gentlemen for their kind advice and criticism. I am also under obligation to the other members of the botanical staff for many courtesies.

Description of the apogamous prothallia

VEGETATIVE MITOSIS

The prothallia which produce sporophytic outgrowths apogamously do so under the influence of artificial culture. The mitoses which occur up to the 30–50-celled stage are exactly similar to those in normal prothallia, but beyond that stage the morphological struc-

ture seems to become influenced by the artificial conditions. The growth becomes very slow, and the cells show a tendency to increase greatly in size, while under normal conditions mitosis would occur before such a size had been reached. Probably for this reason mitotic figures are less frequently met during the growth of such prothallia. As the cell increases in size, its nucleus grows large and the mitotic figure is generally larger than in normal prothallia of this species.

It was not difficult to find stages of mitosis in the vegetative cells, and their comparatively large size facilitated the accurate counting of chromosomes. The resting nucleus contains a delicate reticular structure consisting of a mixture of ragged clumps and slender threads of chromatin. Nucleoli with conspicuous peculiarities of form are always present; sometimes there are two to several isolated round nucleoli scattered irregularly within the nucleus, and sometimes part of them are arranged into a group or groups. They are likely to be mistaken for chromatin nucleoli, but after a close examination of serial stages in the development of the chromosome it is clear that they lie entirely free from the chromatin network and do not seem to contribute any material to the chromosomes by direct transformation. Cell contents beside the nucleus and cytoplasm are not so abundant as in normal cases, the cell cavity consisting largely of vacuoles.

In prophase, the spirem is developed from the chromatin reticulum (*fig. 4*) as described for normal prothallia. The metaphase (*fig. 5*) and anaphase (*figs. 6, 7*) show no peculiar deviation from the typical mitosis. The number of chromosomes is 64 (*fig. 8*) or 66. When two daughter nuclei are reconstructed, a cell plate is laid down between them which finally divides the mother cell into two cells. In this material the binucleate condition was seldom observed, so that it may be claimed that the telophase of mitosis in the vegetative cell is always followed by cell division, and that there is no migration of the nucleus of one cell to an adjacent one.

As stated before, mitosis in the vegetative cells of the normal prothallia and in those reproducing apogamously agree except as to the axis of the spindle, which does not hold any regular relation to the surface of the prothallia, the cell walls being laid down in various directions. This is quite contrary to the condition in normal

prothallia, in which, at least up to the appearance of the archegonium initial, the walls are formed somewhat regularly, being more or less perpendicular or parallel to the surface of the prothallium.

SPERMATOGENESIS

Every apogamous prothallium bears antheridia in profusion, the antheridium initial being formed earlier than in normal prothallia of similar size. The formation of the primary spermatogenous cell, which takes place as in normal prothallia, is studied more readily on account of the comparatively large size. The mitoses from the primary spermatogenous cell to the formation of spermatids and sperms showed no deviation from spermatogenesis in normal pro-thallia. During these mitoses 64 or 66 chromosomes could always be counted. The peculiar structures which were observed within the cytoplasm in the primary spermatogenous cell of the normal prothallia were also present here, but they seemed to be undoubtedly plastids.

The sperms are actively motile and are attracted by 0.01 per cent. solution of sodium malate. From the similarity of the genetic development, morphological structure, and characteristic response to the chemotactic stimulus, it is clear that sperms formed in such pro-thallia can function when conditions permit.

An irregularity in the axis of mitoses was observed during the cell divisions within the antheridium: in most cases the first wall which divides the primary spermatogenous cell vertically is followed by two or three vertical walls parallel to the first, before any trans-verse division takes place.

OOGENESIS

While antheridia and functional sperms are formed in abundance, archegonia are rare in these prothallia. The power of forming archegonia seems to be almost suppressed; and the cushion region where archegonia generally arise is very often covered with antheridia instead of archegonia. In extremely rare cases, however, there appears an archegonium initial, from which a central cell is cut off as in normal prothallia. The central cell either remains without any further division and imbedded below a superficial cell, or develops into canal cells and an egg cell, the projecting neck cells being poorly

developed. The central cell, when it remains undivided, grows to a considerable size, with a corresponding increase in the size of its nucleus, which finally assumes a form similar to that of an egg, but it always appears collapsed. When canal cells and egg are formed, they also appear collapsed. The collapsed appearance of the central, canal, and egg cells is likely induced by the artificial treatment of the prothallia rather than by fixing reagents.

Whether the egg in such a collapsed condition is still capable of fertilization, is questionable, but the writer is inclined to believe that it is incapable of functioning. No case of a sperm having entered an egg was found.

Not only is the formation of archegonia extremely rare, but when formed they are very much belated. In all cases in which

FIG. 1.—Two sections of apogamous prothallia, showing two different stages in the development of archegonia; the shaded regions represent sporophytic outgrowths; *a*, archegonium with central cell; *b*, archegonium with canal cells and egg.

their formation was observed, apogamous sporophytic outgrowths were already in an advanced stage of development (*figs. 1a, b*). Hence it is reasonable to suppose that in the apogamous prothallia a tendency to develop sporophytic outgrowths becomes predominant when the power of forming archegonia becomes weak.

SPOROPHYTIC OUTGROWTHS

As described before, during the early development of prothallia, mitoses occur in the vegetative cells just as in normal prothallia, except that the mitotic figures are comparatively large. The mitoses continue in the vegetative cells and there are organized prothallia of a single cell layer in thickness. The general outline of the prothallia does not show any peculiarities which might be regarded as characteristic of apogamous prothallia as distinct from normal ones.
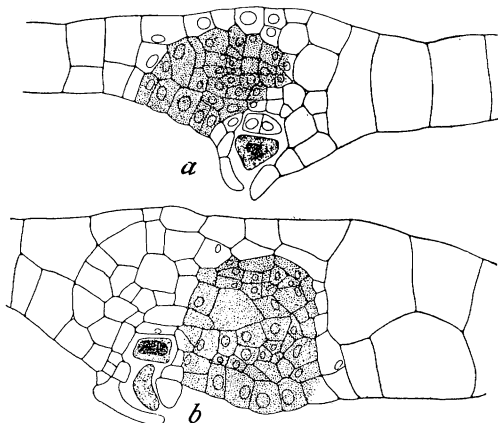
Sporophytic outgrowths begin very early from cells in the region where later the cushion arises, so that the development of the sporophytic outgrowth and the gradual completion of the cushion proceed side by side for a while. When the prothallium has assumed the characteristic heart shape, with a cushion near the sinus and an extensive lateral growth of prothallial tissue on either side, the sporophytic outgrowth is usually in a well-advanced stage. The main features of the formation of this outgrowth and of the gradual completion of the cushion are as follows:

Previous to the formation of the cushion, mitoses take place in rapid succession in the vicinity of the sinus, partition walls always being laid down perpendicular to the surface of the prothallium and parallel to one another, so that the cells formed are very narrow. Some of these mitoses in different stages are represented in *figs. 9–11.* Mitosis continues and cell plates are laid down parallel or oblique to the surface of the prothallium, the ultimate result being the initiation of a thick cushion region (*figs. 12, 13*).

One of the superficial cells in the cushion region begins at once to increase considerably in size, the increase being accompanied by an excessive growth of its nucleus. The nucleus in the resting condition contains a reticulum of ragged clumps and slender threads of chromatin, from which the spirem of the prophase is established (*figs. 14, 14a*). Two or more nucleoli are always present. Successive stages of the mitosis following the early prophase were examined (*figs. 15–17*), which were exactly similar to those of typical mitosis in the vegetative cells of normal prothallia. In the telophase of this mitosis, when the two groups of daughter chromosomes have reached the poles, a little irregularity in the form of the chromosomes is observed, but the number of chromosomes, before they had become aggregated into a mass, was always 64 or 66 (*figs. 18a, b*).

Consequently, it is perfectly clear that, so far as the chromatin is concerned, no change has occurred in the nucleus of these prothallia up to the formation of the superficial cell. In the late telophase a cell plate is laid down perpendicular to the surface of the prothallium, so that there are formed two superficial daughter cells arranged side by side (*fig. 19*).

A number of mitoses follow in the same way, and thus there is

established in a certain region of the superficial layer a group of parallel cells. The increase of the superficial cell is remarkable in apogamous prothallia. In ordinary prothallia, if such a growth in size ever occurs in a superficial cell of the cushion region, it is an archegonium initial; however, the archegonium initial never attains such co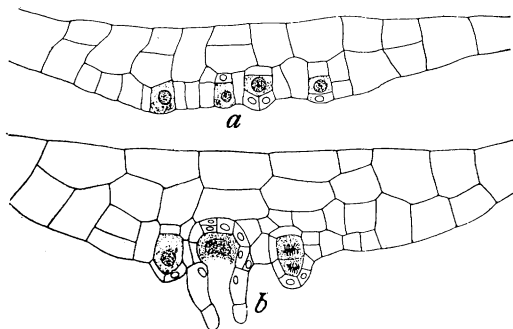nspicuous development, surpassing all the other superficial cells in that region. Moreover, the development of the archegonium generally occurs after the cushion region has extended over a comparatively large area and attained some thickness. On the other hand, the increase in size of the superficial cell in an

FIG. 2.—Two sections of normal prothallia, showing different stages in the development of archegonia.
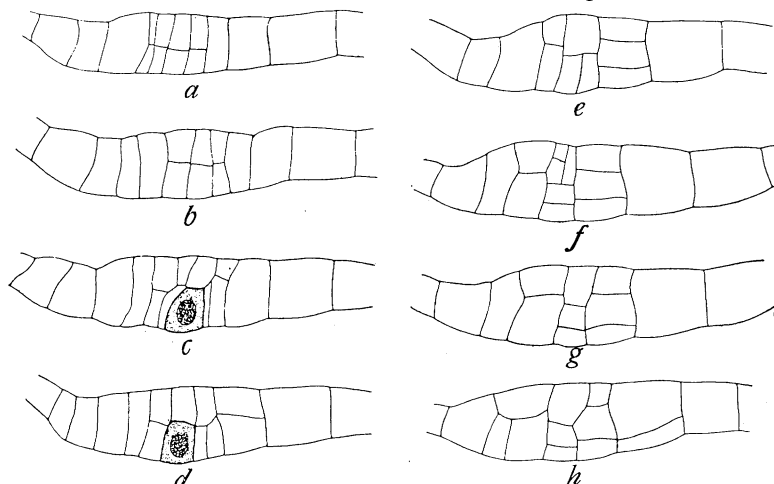
FIG. 3.—Eight successive sections of the same apogamous prothallium showing a single superficial growing to considerable size in a young cushion.

apogamous prothallium commences at an early stage and is associated with the gradual formation of the cushion. The accompanying figures will illustrate the situation. In *fig. 2, a, b* is shown the cushion region, displaying different stages of archegonium development in normal prothallia. *Fig. 3* represents eight successive sections of the same

prothallium, which indicate that a single superficial cell has grown to a considerable size in a young developing cushion region. The remarkable increase in size and the characteristic contents made these cells very conspicuous. Sooner or later, from one of these superficial cells an apical cell is cut off by an oblique wall, and becomes the growing point of a leaf. Sometimes an apical cell appears very early, as shown in *figs. 20* and *21*. During these mitoses, the number of chromosomes is always 64 or 66.

While this process is in progress in the superficial region, cells beneath divide in various directions. Mitoses, of course, occur in various parts during the growth of the prothallia, but they occur more rapidly in the interior region which borders the group of superficial cells whose formation was described above.

Some of the characteristic features that distinguish these interior cells from the other prothallial cells are as follows: they are considerably smaller, probably due to their rapid successive divisions; the nucleus, in spite of the smallness of the cell, is large; the cytoplasmic contents are abundant and the plastids are small and few at this stage.

The mitoses in connection with the formation of this group of interior cells were studied. The general aspect of the mitotic figures in successive stages and the behavior of the chromosomes in these mitoses were similar to those of typical mitosis in the vegetative cells of normal prothallia. It was interesting to notice, however, that the narrowness of the cell cavity and the largeness of the nucleus showed a remarkable resemblance to the condition observed during the development of normal embryos later than the 32-celled stage (*figs. 22–29*). In telophase the number of chromosomes was always 64 or 66 (*fig. 27*).

The mitoses in the groups of superficial and interior cells continue as described above, and there is formed a structure which is in direct connection with the prothallial cells, the structure that later becomes an independent sporophyte. *Figs. 28* and *28a* represent the structure of the sporophytic outgrowth at a certain stage in the course of its development. One apical cell which is already differentiated in the structure is not shown in this section.

Thus the structure of the sporophytic outgrowth is brought about

apparently by the cooperation of the mitoses of two different regions, one of which is a single superficial cell that has attained a conspicuous size, and the other the vegetative cells immediately beneath. If we trace further back the origin of the cells of these regions, they are found to be descendants from the vegetative cell or cells whose sister cells have organized the cushion region. It was impossible to detect a distinct period in which any change had occurred in the chromatin condition up to this development of the sporophytic outgrowth; practically the nucleus of the gametophyte has become directly the nucleus of the sporophytic outgrowth, without any nuclear fusion.

The sporophytic structure develops with repeated mitoses of the same sort as was described before: a leaf and a stem apex are developed from two apical cells which have appeared one after the other; a root initial is organized endogenously (*fig. 29*); scalariform vessels appear in the tissue connecting the leaf and stem origins with the root initial; and finally there is developed an independent leafy sporophyte.

From the foregoing it is clear that there is established a sporophyte with the haploid or x number of chromosomes in *Nephrodium molle*. This is the first instance yet known in plants, in which a sporophyte with the haploid number of chromosomes has been described.

Whether the sporophyte thus formed may produce spores has not yet been determined.

Discussion of cytological phenomena

APOGAMY

Since the first discovery of apogamy in *Pteris cretica*, instances of apogamy in pteridophytes have steadily increased until the phenomenon is now known in about fifty forms. No cytological studies were recorded until the appearance of the papers of STRASBURGER and of FARMER and DIGBY, already cited, but there had appeared several cytological studies of the apogamous development of the embryo in spermatophytes.

Apogamy (parthenogenesis and vegetative apogamy) is now known in spermatophytes for *Allium odorum* (TRETJAKOW **69,** HEGEL-MAIER **28**), Balanophora (TREUB **70,** LOTSY **39**), *Antennaria alpina*

(JUEL **30, 31**), several species of Alchemilla (MURBECK **44, 45, 46**;
STRASBURGER **66**), *Thalictrum purpurascens* (OVERTON **53, 54**), *Gne-tum Ula* (LOTSY **40**), several forms of Taraxacum (RAUNKIAER **57**;
MURBECK **47**; JUEL **32, 33**), a number of species of Hieracium
(OSTENFELD **50, 51**; MURBECK **47**; ROSENBERG **59**), and *Wik-stroemia indica* (WINKLER **75, 76**). Among these contributions
those of JUEL, MURBECK, OVERTON, STRASBURGER, WINKLER, and
ROSENBERG present some very interesting cytological data.

JUEL (**30, 31**) made a comparative study of the parthenogenetic
Antennaria and the normally fertilized *A. dioica*. In the latter a
tetrad is formed from a megaspore mother cell, with the usual reduction
of chromosomes, and the embryo sac is developed from one of the
megaspores. There is a typical synapsis preceding a heterotypic
mitosis, and the embryo sac is normal. The reduced number of
chromosomes is 12–14 in the pollen mother cell and 20–24 in the
integument. In the parthenogenetic *Antennaria alpina*, not only is
the tetrad suppressed, but there is no trace of heterotypic and homo-
typic mitosis in the embryo sac. The number of chromosomes is
40–50 in the embryo sac and 45–50 in the integument. There is thus
no reduction of the chromosomes during the formation of the embryo
sac, and the egg retains the sporophytic number.

MURBECK (**44, 45, 46**) studied eight species of Alchemilla, chief
attention being paid to the parthenogenetic *A. alpina*. In the
parthenogenetic species of EUALCHEMILLA, he found that the embryo
sac always developed from one megaspore of the tetrad produced
from the megaspore mother cell through two successive mitoses, in
which there seemed to be no evidence of a reduction of chromosomes.
The number of chromosomes in these divisions is approximately 32–48,
and this number is retained in the egg nucleus and the other nuclei of
the embryo sac. He reports that the embryo of *A. sericata* is produced
from a synergid. In the normal species *A. arvensis*, belonging to the
section APHANES, he finds in the pollen mother cell 16 chromosomes—
the reduced number. Two years later, MURBECK (**47**) in a short paper
announced that embryos in *Taraxacum vulgare* Raunk. and *speciosum*
Raunk. and *Hieracium grandidens, serratifrons*, and subsp. *crispa-tum* develop from flowers whose stamens have been removed, but
he did not make any cytological studies. JUEL (**32, 33**) discovered a

peculiar development of the embryo sac in the parthenogenetic *Taraxacum officinale*. The species produced pollen with a normal reduction of chromosomes, and 13 bivalent chromosomes were present in the heterotypic mitosis; but the megaspore mother cell undergoes a single mitosis and there are formed two daughter cells, the lower one of which develops into the embryo sac. This mitosis passes through the synapsis and leptonema stages as usual, but the heterotypic figure is not organized; it is a typical vegetative one with the univalent 24–26 chromosomes. The nuclear divisions in the embryo sac were not followed by JUEL, but, accepting the results of MURBECK, he believed that the egg nucleus retained 26 chromosomes—the sporophyte number.

OVERTON (**53, 54**) found a normal reduction in the pollen mother cells of *Thalictrum purpurascens* and showed that the number of chromosomes is 24 for the sporophyte and 12 for the gametophyte. The development of the embryo sac is of two types. In some cases a tetrad is formed from a megaspore mother cell, with all the phenomena of a reduction division; the lowest cell develops into the embryo sac. But many embryo sacs are formed in a different way. The first mitotic figure in the megaspore mother cell is not heterotypic, and shows 24 univalent chromosomes; and the same number is counted in the second division in the parthenogenetic embryo. He concludes that the sporophytic number (24) remains unchanged in the embryo sac in this case, and that the egg nucleus with the sporophytic number develops into the embryo parthenogenetically.

STRASBURGER (**66**) made an extensive study of numerous species of Alchemilla § EUALCHEMILLA, the group which furnished the material for MURBECK'S important discoveries. Most of the forms in EUALCHEMILLA develop normal pollen and a reduction division was found both here and in *Alchemilla arvensis* of § APHANES. In the heterotypic mitosis in the pollen mother cell, STRASBUGRER found 32 bivalent chromosomes, which MURBECK counted as 16. In the embryo sac development in apogamous species the two characteristic divisions of sporogenesis are cut out and no tetrad is formed. The nucleus of the megaspore mother cell emerges from synapsis with the sporophytic number of univalent chromosomes, and the ensuing division is typically vegetative and not heterotypic. The nuclei of the embryo sac thus contain the sporophytic number and parthenogenetic develop-

ment of the egg takes place. STRASBURGER regards the parthenoge-
netic tendency of EUALCHEMILLA as associated with excessive muta-
bility, which has weakened sexuality so that the process of fertilization
is being displaced by apogamy.

WINKLER (**75, 76**) reports an interesting case of apogamy in
Wikstroemia indica. He observed that seeds are produced apoga-
mously, in spite of the fact that some pollen matures. The apogamous
development of the embryo was proved by castration experiments.
He describes a peculiar phenomenon in the cells of the tapetal layer,
which usually contain two to six nuclei. These nuclei fuse into a
huge nucleus, whose mitotic figure often shows over 100 chromosomes;
but usually he counted 52 chromosomes. Although most of the
pollen does not reach maturity, a tetrad division with reduction is
present in sporogenesis, and in the heterotypic figure there are 26
bivalent chromosomes. The micropyle of the ovule is closed by the
elongation of the inner wall of the ovary during the formation of
embryo sac, which undoubtedly may have some relation to the apoga-
mous development of the embryo. A megaspore mother cell in this
form becomes directly an embryo sac, with the entire suppression of
tetrad division. His material was not sufficient for a cytological
study of the mitoses, and consequently he was unable to determine
the entire absence of a reduction division in the embryo sac, but it
seemed very likely that the egg which develops parthenogenetically
may retain the sporophytic number of chromosomes.

ROSENBERG (**59**) presents the result of cytological studies on six
species of Hieracium. He took up the species of Hieracium in which
apogamy was proved by the experimental studies of OSTENFELD (**52**),
and traced out their nuclear details. In *Hieracium excellens* the
nucleus of the pollen mother cell, after it has come out from synapsis,
presents a heterotypic figure with 17 bivalent chromosomes, but
often with an irregularity in the number of the bivalent and univalent
ones. When daughter halves of the bivalent chromosomes separate
and become grouped to form the daughter nuclei, the univalent ones
are left behind in the cytoplasm, as he had already observed in Dro-
sera (**58**). In *H. flagellare* a normal heterotypic mitosis takes place
in the pollen mother cell, the reduced number of chromosomes being
21. In these two species, the embryo sac develops after a tetrad divi-

sion, as in normal cases; in rare cases the tetrad division with reduc-
tion is entirely cut out. The egg retaining the sporophytic number
of chromosomes then develops the embryo parthenogenetically. But
in the majority of cases he reports that while the normal development
of the embryo sac is proceeding, an embryo sac from a cell quite near
the tetrad (in *H. aurantiacum*), or in the integument, or in the chalazal
region commences to develop. The normal embryo sac is then
destroyed sooner or later by the encroaching embryo sac of vegetative
origin. This development of an embryo sac from the nucellus is a
new case, entirely different from those known in Funkia, Coelebogyne,
Citrus, Opuntia, and *Alchemilla pastoralis*, for in the latter cases the
embryo is produced directly from the nucellus, instead of through an
intercalation of embryo sac formation, and hence the embryo is a
vegetative bud from the sporophyte and entirely independent of
gametophytic activities.

The papers of STRASBURGER and of FARMER and DIGBY, which
are the latest contributions to the cytology of apogamy among pterido-
phytes, were briefly reviewed in the first part of this paper.

Summarizing the cytological facts in connection with apogamy
among spermatophytes, as interpreted by different investigators, it
seems evident that apogamy is closely associated with the suppression
of sporogenesis in the megaspore mother cell. This necessarily results
in no change in the chromosome number in the nucleus of the sporo-
phytic generation, yet a structure is developed with the morphology
of the gametophytic generation. Thus the embryo sac will contain
the usual number of nuclei grouped in the typical manner, but these
nuclei have the sporophytic number of chromosomes. From the facts
of apospory, it seems probable then that the development of a game-
tophyte may result from an interference with the normal life-history
and under conditions favorable to the gametophyte, even though
the nuclei retain the sporophytic number of chromosomes.

If the doubling of chromosomes is that result of fertilization
necessary to start the sporophyte generation, it is no surprise that either
an egg with the sporophytic number or a vegetative nucleus with the
same number may develop a sporophyte. In all the foregoing cases
of apogamy this seems to be the situation.

The case of vars. *polydactyla* studied by FARMER and DIGBY is

an instance in which the apogamous development of the sporophyte is
not preceded by apospory.   The prothallia in these forms are pro-
duced after normal sporogenesis and consequently they contain the
gametophytic number of chromosomes.   The authors claim to have
found in certain vegetative cells the fusion of two nuclei, one of which
has entered the cell from an adjacent one, and that the sporophyte
develops from the region of the prothallium where this fusion occurs.
They regard this fusion of two vegetative nuclei as a substitute for
normal fertilization.   A similar instance of the fusion of two vegetative
nuclei is given by BLACKMAN (**4**) for Phragmidium, who regards (**4,
5**) the process as a reduced form of fertilization.   The conjugated
nuclei divide simultaneously through a long series of nuclear divisions,
from the formation of aecidiospores to that of teleutospores, where
the last pairs unite to form the single fusion nuclei within the teleuto-
spores.   There is much evidence that the period in the life-history
characterized by the presence of the paired nuclei represents a sporo-
phytic phase.   Thus the fusion of the two nuclei in vars. *polydactyla*
and the pairing condition of the two nuclei in Phragmidium may
support as a working hypothesis the assumption that a nucleus with
the sporophyte number of chromosomes is necessary for starting the
sporophyte generation.

The condition shown in the apogamous Nephrodium is entirely
different from anything yet recorded for plants.   The prothallia are
developed after normal sporogenesis and their nuclei retain the
gametophytic number of chromosomes.   The sporophyte then appears
as a vegetative outgrowth from the prothallium, without any visible
change in the nuclei, so that there is established a sporophyte with
the gametophytic number of chromosomes.   FARMER and DIGBY (**24**)
have suggested that the number of chromosomes, approximately
60, which is found throughout the life-cycle in *Lastrea pseudo-mas*
var. *cristata apospora* may be the gametophytic number in the type
species, that is 72, and that this variety might have arisen from normal
prothallia of the type species through apogamy.   WILLIAMS (**74**)
gives an instance of true apogamous development of the egg in
Dictyota, which is as yet the only type among algae where the nuclear
conditions of apogamy are known.   In WILLIAMS' cultures the ger-
mination of the unfertilized egg with 16 chromosomes, the gameto-

phyte number, presented many irregularities in the segmentation divisions and most of the young embryos died after four divisions. Dictyota, then, cannot be regarded as furnishing an instance similar to Nephrodium, since the apogamous developments are abortive and it was not determined whether the structures were sporophytic or gametophytic in nature.

As regards the application of the terms apogamy and parthenogenesis in the various cases observed, STRASBURGER'S principle (**66**) is based upon the number of chromosomes contained in the embryo asexually produced; that is, the asexual development of an embryo from the gametophyte with the diploid number of chromosomes, no matter whether it originates from an egg or a vegetative cell, he calls apogamy; and he regards an unfertilized egg with the diploid number as a vegetative cell. He would restrict the term parthenogenesis to the asexual development of an egg, with the haploid number of chromosomes, and with the capability of being fertilized. WINKLER'S (**76**) view is different. He applies the term parthenogenesis to the case of an asexual development of an egg cell, no matter whether it be haploid or diploid, and he proposes to restrict the use of the term apogamy to cases in which the sporophyte is formed as a vegetative outgrowth from the gametophyte. FARMER and DIGBY'S (**24**) terminology, though not similar, resembles WINKLER'S. The terms euapogamy and parthenogenesis are applied respectively to cases of asexual development of the sporophyte from vegetative cells and from an egg cell of a prothallium produced aposporously; and to the case where a sporophytic outgrowth is preceded by the fusion of two vegetative nuclei they apply the term pseudo-apogamy. The apogamy in Nephrodium, therefore, would be called apogamy by WINKLER, euapogamy by FARMER and DIGBY, and represents no category given by STRASBURGER.

ALTERNATION OF GENERATIONS

Since HOFMEISTER'S investigations we have known that the life-history of most plants involves a regular alternation of sexual and asexual generations. The subject has been discussed by many authors, such as CELAKOWSKY (**13, 14, 15**), SACHS (**60**), BRAUN (**10**), PRINGSHEIM (**55, 56**), VINES (**72**), DE BARY (**2**), BOWER

(7, 8, 9),VAIZEY (71), STRASBURGER (65, 67), BEARD (3), CAMPBELL (11, 12), SCOTT (61), LANG (36, 37), KLEBS (34, 35), HARTOG (27), COULTER (18), DAVIS (19, 20), WILLIAMS (73), BLACKMAN (4, 5), WOLFE (77), LOTSY (41, 42), GRÉGOIRE (25), CHAMBERLAIN (16), CHRISTMAN (17), OLTMANNS (49), HARPER (26), YAMANOUCHI (80), and many others.

Among thallophytes no generalization for the whole group is possible at present, partly because of extreme diversity, and partly on account of the meagerness of our knowledge regarding the life-cycle of the majority of the forms. Different opinions are held concerning the nature of the phenomena in various forms, and some even question the existence of an alternation of generations. However, it is now being gradually established by actual investigation, and quite recently cytological proof has been obtained from several forms, as Dictyota, Phragmidium, Nemalion, Polysiphonia, and some others.

Pteridophytes and bryophytes have been regarded as the best illustrations. Discussion in connection with the pteridophytes has not been in reference to the existence of alternation, but has centered about the question whether it is to be interpreted as of antithetic or homologous origin.

These two views represent different conceptions as to the origin of the sporophyte. Those who advocate the theory of antithetic origin regard the sporophyte of pteridophytes as a gradual elaboration from the zygote of some aquatic algal ancestor, a new phase having thus been intercalated in the life-history. This view was first clearly stated by CELAKOWSKY (13, 14). BOWER (7, 8, 9) supported it and endeavored to explain it as an adaptation to external conditions. STRASBURGER (65), restating the position in terms about identical with BOWER'S, based the theory upon nuclear details. Those who maintain the theory of homologous origin consider that the sporophyte arose as a modification of the gametophyte, and not as a new structure. PRINGSHEIM (55, 56), and more recently SCOTT (61), LANG (36, 37), COULTER (18), and others advocate the homologous theory. This theory is largely based upon the phenomena of apogamy and apospory and also to a certain extent upon experiments in regeneration.

When these two theories were proposed, cytological investigations had not yet developed, and even LANG'S admirable work did not

touch any cytological particulars.   Since the announcement of STRAS-
BURGER'S view of the antithetic origin of alternation, the first to be
based on cytological details, chief attention has been directed by many
workers to the behavior of chromosomes during the reduction division
in the normal life-cycle.   As to the results of such accumulated studies,
the various views are not readily grouped.   However, the majority of
cases confirms the view that the periodic reduction of chromosomes
is necessary; in other words, the gametophyte with the x number
of chromosomes is entirely distinct from the sporophyte with the $2x$
number, and the transition from one generation to the other is marked
by the reduction of chromosomes in sporogenesis and the doubling
of chromosomes in fertilization, in connection with which the pre-
dominant characteristics of one generation are entirely lost and the
potentialities of forming the other generation are regained.

As stated above, the cytological work on apogamy and apospory has
been chiefly among flowering plants; and quite recently our knowledge
concerning these phenomena in ferns was extended by the contribution
of FARMER and DIGBY (24) on Lastrea, Athyrium, and Scolopendrium,
and of STRASBURGER (68) on Marsilia.   According to these investi-
gations, apogamy, whatever its cause may be, is always preceded
either by apospory or the fusion of two vegetative nuclei, which seems
to favor the view that the $2x$ number of chromosomes is necessary to
establish the sporophyte.   Apogamy and apospory, which have been
the chief argument for the theory of homologous origin, now seem to
support the theory of antithetic origin.

As a matter of fact, the nuclear condition in Nephrodium in the
normal life-cycle confirms the antithetic theory; but apogamy in
Nephrodium introduces a new situation.   In this case apogamy
is preceded neither by apospory nor fusion of vegetative nuclei, but
the sporophytes are developed with the haploid number of chromo-
somes.   If it might be questioned whether the situation in Nephro-
dium—in which the nucleus of the gametophyte with the x number
can establish the sporophyte—may favor the idea of homologous
origin, it must be remembered that we have such abnormal cases of
apogamy and apospory in flowering plants, where the embryo sac
(probably gametophytic) does not contain the characteristic x number
of chromosomes, but always the $2x$ number.

Conclusion

According to the present interpretation of the value of chromosomes, in *Nephrodium molle* Desv. there is present an antithetic alternation of generations marked by the number of chromosomes. Apogamy in Nephrodium presents a new situation, where the sporophyte is developed with the haploid number of chromosomes. This seems to be an abnormal case, but it must be admitted that it shows that the number of chromosomes is not the only factor which determines the characters of the sporophyte and gametophyte.

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NOTE.—After this paper was in type, STRASBURGER'S "Chromosomenzahlen, Plasmastrukturen, Vererbungsträger und Reduktionsteilung" (Jahrb. Wiss. Bot. **45**:479–568. *pls. 1–3.* 1908) appeared. It not only presents results of investigations on *Lilium Martagon* dealing with the chromosome situation during embryo sac formation and pollen tube development, but it also contains voluminous data concerning plasma structure, chromosomes as bearers of hereditary characters, and the phenomena of the reduction division, the discussion being based upon works of various invesitgators of both plant and animal cells.

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EXPLANATION OF PLATES IX AND X

The figures were drawn with the aid of an Abbé camera lucida, under Zeiss apochromatic obj. 1.5^{mm} N. A. 1.30, combined with compensating ocular 18; except *figs. 9, 10, 11, 12, 13, 14, 20, 21, 28a* drawn with compensating ocular 4; and *figs. 28, 26,* and text cuts drawn under combination of dry obj. 4^{mm} and ocular 4. The plates and text cuts are reduced to one-half the original size.

FIGS. 1–3 are in the text.

PLATE IX

FIG. 4.—Nucleus of vegetative cell of an apogamous prothallium; spirem formed; two nucleoli present.

FIG. 5.—Late prophase; long slender chromosomes present, previous to their arrangement in an equatorial plate.

FIG. 6.—Metaphase; two daughter halves of each chromosome just separated.

FIG. 7.—Late anaphase; daughter chromosomes grouped at two poles.

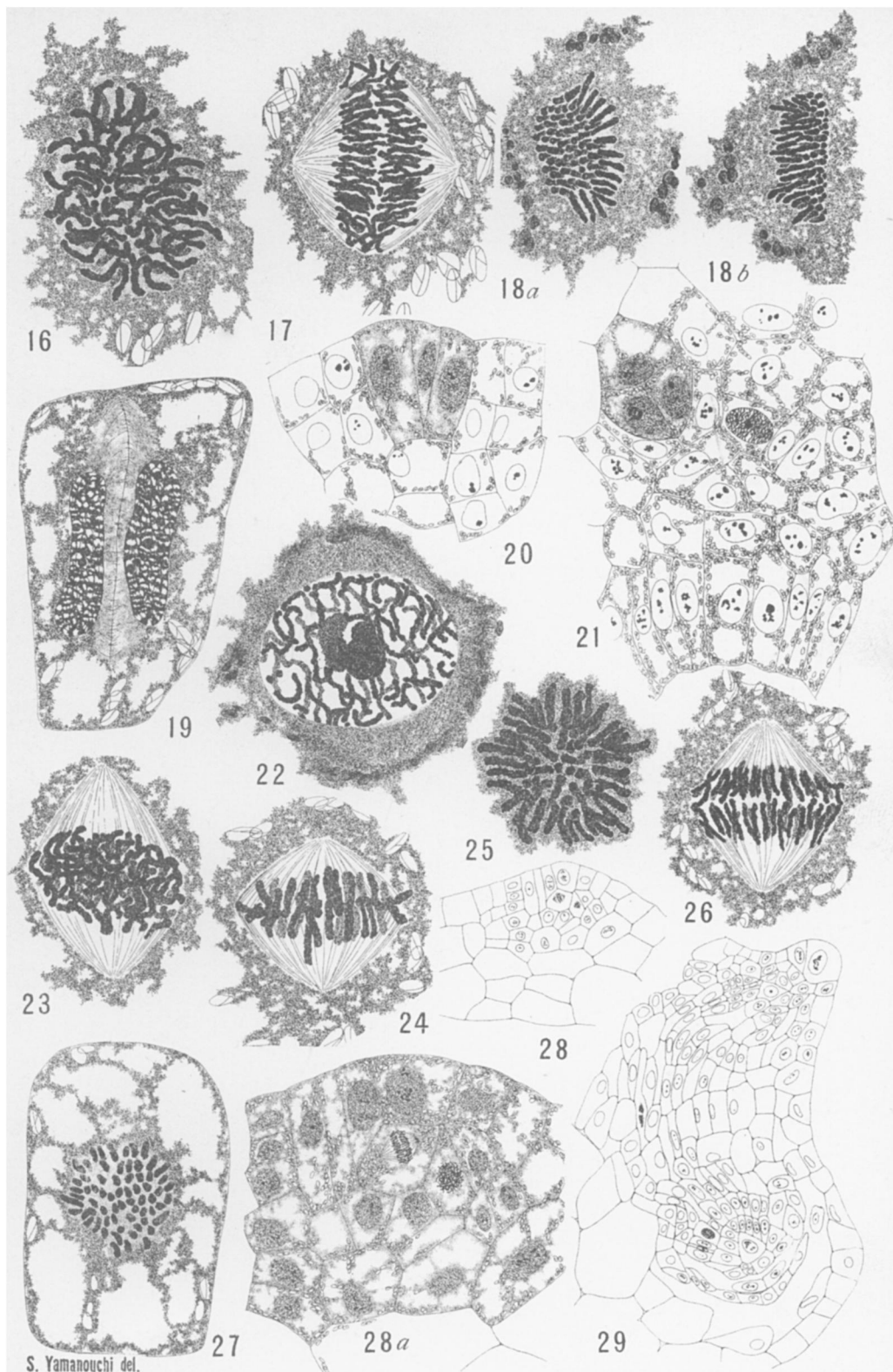FIG. 8.—Polar view of late anaphase; 64 chromosomes present in a group.

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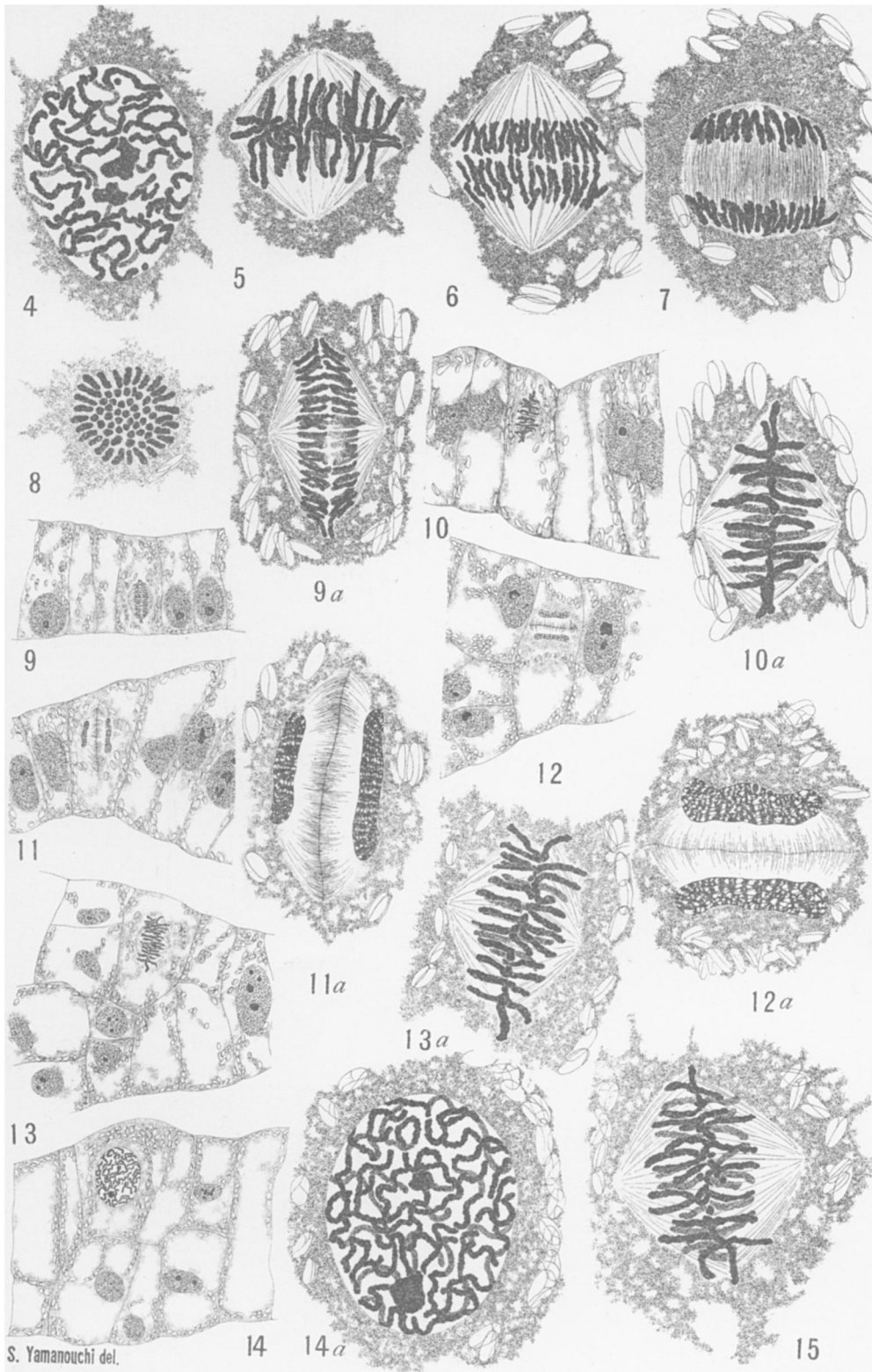
Fɪɢ. 9.—Section through region where later a cushion arises; narrow cells parallel.

Fɪɢ. 9*a*.—Nucleus in metaphase from previous figure, under higher magnification.

Fɪɢ. 10.—Section through region forming cushion; next stage after that shown in *fig. 9*.

Fɪɢ. 10*a*.—Nucleus in late prophase from previous figure, under higher magnification.

Fɪɢ. 11.—Section through region forming cushion; later stage than that shown in *fig. 10*.

Fɪɢ. 11*a*.—Telophase of nucleus shown in previous figure, under higher magnification.

Fɪɢ. 12.—Section including cushion region; next stage after that given in *fig. 11*.

Fɪɢ. 12*a*.—Mitotic figure in telophase, shown in *fig. 12*, under higher magnification; cell plate laid down parallel to surface of prothallium.

Fɪɢ. 13.—Section of cushion region; a nucleus within superficial cell in metaphase.

Fɪɢ. 13*a*.—Nucleus in metaphase, shown in the previous figure, under higher magnification.

Mitosis in conspicuous superficial cell

Fɪɢ. 14.—One of the sections of the cushion region shown in *fig. 3* (text); one superficial cell has increased considerably in size; nucleus in prophase.

Fɪɢ. 14*a*.—Nucleus in prophase, shown in *fig. 14*, under higher magnification; several ends of the spirem seen are cut sections; two nucleoli present.

Fɪɢ. 15.—Late prophase, showing long slender chromosomes before arrangement in an equatorial plate.

PLATE X

Fɪɢ. 16.—Polar view of the late prophase shown in preceding figure.

Fɪɢ. 17.—Metaphase; two daughter halves of each chromosome just separated.

Fɪɢs. 18*a*, 18*b*.—Two groups of daughter chromosomes in late metaphase of a nucleus cut obliquely into two sections; 66 chromosomes present.

Fɪɢ. 19.—Telophase; cell plate, vertical to surface of prothallium, laid down to cut superficial cell into two daughter cells.

Fɪɢ. 20.—Portion of section cut through cushion region; three superficial cells drawn showing contents, middle one of which is cut obliquely as an apical cell.

Fɪɢ. 21.—Surface view of a stage similar to that shown in preceding figure; the three superficial cells in question represented by drawing all the contents.

Mitosis in interior cells directly beneath superficial cells

Fɪɢ. 22.—Nucleus containing a spirem; visible ends of spirem are cut sections.

FIG. 23.—Prophase; chromosomes irregularly crowded in a mass.

FIG. 24.—Late prophase, showing chromosomes before their arrangement in an equatorial plate.

FIG. 25.—Polar view of metaphase, where L-shaped chromosomes are arranged in an equatorial plate; vertical arms of L's are visible as dots and lateral arms as radiating lines.

FIG. 26.—Late metaphase; two daughter halves of each chromosome have just separated.

FIG. 27.—Early telophase, showing polar view of a group of daughter chromosomes; 66 chromosomes present.

FIG. 28.—Section of cushion region where a sporophytic structure has been worked out.

FIG. 28a.—Approximate portion that belongs to the sporophytic structure shown in the preceding figure, under higher magnification; difference of size of cells in prothallial region and sporophytic structure shown.

FIG. 29.—Section through sporophyte apogamously produced, in a considerably later stage; one of the two apical cells seen is a leaf initial, the other a root initial; apical cell of stem not drawn in this section; no structure standing for a foot is present.